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Comparison of a Newly Developed Automated and Quantitative Hepatitis C Virus (HCV) Core Antigen Test with the HCV RNA Assay for Clinical Usefulness in Confirming Anti-HCV Results▽

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Hepatitis C virus (HCV) is a global health care problem. Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies as a screening test with serum samples. Recombinant immunoblot assays are used as supplemental tests and for the final detection and quantification of HCV RNA in confirmatory tests. In this study, we aimed to compare the HCV core antigen test with the HCV RNA assay for confirming anti-HCV results to determine whether the HCV core antigen test may be used as an alternative confirmatory test to the HCV RNA test and to assess the diagnostic values of the total HCV core antigen test by determining the diagnostic specificity and sensitivity rates compared with the HCV RNA test. Sera from a total of 212 treatment-naive patients were analyzed for anti-HCV and HCV core antigen both with the Abbott Architect test and with the molecular HCV RNA assay consisting of a reverse transcription-PCR method as a confirmatory test. The diagnostic sensitivity, specificity, and positive and negative predictive values of the HCV core antigen assay compared to the HCV RNA test were 96.3%, 100%, 100%, and 89.7%, respectively. The levels of HCV core antigen showed a good correlation with those from the HCV RNA quantification ($r = 0.907$). In conclusion, the Architect HCV antigen assay is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective, and applicable as a screening, supplemental, and preconfirmatory test for anti-HCV assays used in laboratory procedures for the diagnosis of hepatitis C virus infection.

Hepatitis C virus (HCV) was first recognized in 1974 as a non-A, non-B hepatitis virus (NANBH) and first identified in 1989 using molecular methods, but to date, the virus has never been visualized or grown in cell culture (7, 22). HCV is a positive-strand RNA virus that belongs to the family *Flaviviridae* (14). HCV is a global health care problem, and the World Health Organization (WHO) estimates that at least 170 million people (3% of the world's population) are infected with HCV worldwide (30).

Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by the enzyme immunoassay (EIA) or chemiluminescence immunoassay (CLIA) of serum samples. The anti-HCV assay is used as a screening test. Recombinant immunoblot assays are used as supplemental tests and for the final detection and quantification of HCV RNA in confirmatory tests. Three different generations of anti-HCV test kits have been developed. The first-generation HCV EIA detects only antibodies against the nonstructural region 4 (NS4) with recombinant antigen c100-3 (12). With the development of second-generation tests, additional antigens from the core region (c22-3), the NS3 region (c33c), and a part of c100-3 (5-1-1) from the NS4 region can be used (8). The third-generation EIA anti-HCV test currently used includes an additional antigen from the NS5 region and a reconfiguration of the core and NS3 antigens (29).

Anti-HCV assays have several disadvantages, such as a high rate of false positivity, a lack of sensitivity of detection in the early window period of 45 to 68 days after infection, the inability to distinguish between acute (ongoing active, viremic), past (recovered), and persistent (chronic) infections, and a possibility of false negativity with samples from immunocompromised patients, who may not have an adequate antibody response (9, 17, 19, 21). Recombinant immunoblot assays, types of EIAs, also have several disadvantages, such as being difficult to perform and having a high percentage of indeterminate results and a high cost. Therefore, these anti-HCV assays are not often used in developing countries or in routine diagnostic laboratory procedures (10). The HCV RNA assay is a reliable method but needs technical skill and may also result in false positivity because of contamination, and it is time intensive and more expensive (16).

In this study, we aimed to compare the HCV core antigen (HCV Ag) test with the HCV RNA assay for confirming anti-HCV results to determine whether the HCV Ag test may be used as an alternative confirmatory test to the HCV RNA test and to assess the diagnostic values of the total

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TABLE 1. Summary of HCV Ag, HCV RNA, and anti-HCV test results

HCV Ag test result	No. of samples with the following HCV RNA load (no. of anti-HCV-positive samples/total no.):			
	Not detected	$<10^5$ IU/ml	$>10^5$ IU/ml	Total
Nonreactive	52 (38/52)	6 (1/6)	0 (0/0)	58 (39/58)
Reactive	0 (0/0)	104 (104/104)	50 (50/50)	154 (154/154)
Total	52 (38/52)	110 (105/110)	50 (50/50)	212 (193/212)

HCV Ag test by determining the diagnostic specificity and sensitivity rates compared with the HCV RNA test.

MATERIALS AND METHODS

Patients and samples. Serum samples were collected from patients at low risk for hepatitis C virus infection who were referred to the Department of Microbiology, Konya Education and Research Hospital (Konya, Turkey), between October 2010 and April 2011.

Ethical approval. Ethical approval was provided by the Ethics Committee of Meram Medical School, Selcuk University (Konya, Turkey). Patients provided both verbal and written consent prior to their participation.

Anti-HCV screening test. All sera were analyzed using the commercially available anti-HCV automated CLIA system for the detection of immunoglobulin G (IgG) antibodies to the hepatitis C virus. The Architect anti-HCV assay using the Architect i2000SR system (Abbott Laboratories, Diagnostics Division, Abbott Park, IL) uses automated chemiluminescent detection technology (Chemiflex), and the reactive component contains recombinant antigens representing the core and the NS3 and NS4 proteins HCr43 and c100-3. HCr43 is composed of two noncontiguous coding regions of the HCV genome sequence, the 33c and core regions; c100-3 is a recombinant HCV protein within the putative nonstructural (NS3 and NS4) regions of HCV. The anti-HCV screening assay was performed according to the manufacturer's instructions. The Architect i2000SR was used in the Department of Microbiology, Selcuklu Medical School, Selcuk University (Konya, Turkey).

HCV Ag and HCV RNA confirmatory tests. The Architect HCV Ag assay was performed using the automated Architect i2000SR CLIA system (Abbott Laboratories, Diagnostics Division, Abbott Park, IL). The Architect HCV Ag assay uses a two-step chemiluminescent microparticle immunoassay technology for the quantification of the HCV Ag in human serum or plasma samples. The sample volume required is approximately 110 μl, and the total assay time is approximately 36 to 40 min. The cutoff value is 3.00 fmol/liter (0.06 pg/ml); thus, samples with values of <3.00 fmol/liter are considered nonreactive, samples with values of ≥3.00 fmol/liter are considered reactive, and samples with values of ≥3.00 fmol/liter and <10.00 fmol/liter are retested in duplicate. If both retest values indicate that the specimen is nonreactive, the specimen is considered nonreactive for HCV Ag. If one or both of the duplicates have values of ≥3.00 fmol/liter, the specimen is considered repeatedly reactive (16, 23).

The molecular HCV RNA assay is a confirmatory test (Qiagen, Hilden, Germany) with a lower detection limit of 20 IU/ml which uses a reverse transcription-PCR (RT-PCR) method using Rotor Gene 6000 real-time analysis (Corbett Research, Sydney, Australia). The RNA was extracted from serum samples using a spin column method (QIAamp viral RNA minikit; Qiagen). The HCV Ag test was performed in the Department of Microbiology, Selcuklu Medical School, Selcuk University (Konya, Turkey), and the HCV RNA assay was performed in the Department of Microbiology, Konya Education and Research Hospital (Konya, Turkey), according to the manufacturer's instructions.

HCV genotype detection. A reverse hybridization method and kit (Versant HCV genotype assay, a line probe assay[LiPA], version 2.0; Bayer HealthCare LLC, Tarrytown, NY) was used for hepatitis C virus genotype assay. The test is mainly based on biotinylated DNA, generated by RT-PCR amplification of the 5′ untranslated region (5′ UTR) of HCV RNA, hybridized to immobilized oligonucleotide probes.

Statistical analysis. Statistical analysis was carried out with SPSS statistical software, version 14.0 (SPSS Inc., Chicago, IL), for Windows, and a P value of <0.05 was considered to be significant. The agreement, sensitivity, specificity, positive and negative predictive values, Spearman's correlation coefficient, and regression analysis tests were used for statistical analysis of the data. We also analyzed the ratio between HCV RNA (in IU/ml) and HCV Ag (in fmol/liter) by the nonparametric Mann-Whitney U test in the Abbott anti-HCV assay results separated according to the HCV RNA median.

RESULTS

A total of 212 patients provided serum: 122 (57.5%) women and 90 (42.5%) men. Their mean age ± standard deviation was 59 ± 14.5 years (57.7 ± 16.2 years for women and 60.7 ± 11.9 years for men). All the tested sera were obtained from treatment-naive patients. All HCV RNA-positive sera were found to be HCV genotype 1b.

Among the 212 specimens tested, all 52 cases in which HCV RNA was not detected were also nonreactive for HCV Ag. For all samples with an HCV RNA-negative result, a new sample was retested twice, 2 and 4 weeks later, and all of them were again found to be negative. HCV Ag was found to be negative in only 6 out of 160 HCV RNA-positive samples; these samples had low-level viremia (5 samples with HCV RNA at levels between 75 and 249 IU/ml and 1 sample with 6,562 IU/ml). All the HCV Ag-reactive cases ($n = 154$) were also found to be positive with the anti-HCV test. Of all the 38 false-positive anti-HCV results with the Architect anti-HCV assay, 28 had sample value-to-cutoff (S/CO) ratios of less than 6 (22 had S/CO ratios between 1.3 and 2.54, 6 had S/CO ratios between 3.02 and 5.47), 7 had S/CO ratios of between 6 and 8, and only 3 had S/CO ratios of between 12 and 16. A summary of HCV Ag, HCV RNA, and anti-HCV test results is presented in Table 1.

The distribution of positive and negative results with the HCV Ag and HCV RNA tests is presented in Table 2.

The diagnostic sensitivity, specificity, and positive and negative predictive values of the HCV Ag assay compared to the HCV RNA test were 96.3%, 100%, 100%, and 89.7%, respectively (Table 2).

Figure 1 illustrates individual HCV Ag and RNA concentrations, showing a good correlation between them ($r = 0.864$; $P = 0.0001$).

A regression analysis of these 212 specimens yielded the equation $y = 0.81x - 1.435$ with a correlation coefficient of 0.864, where y is the concentration of HCV Ag in $\log_{10}$ pico-

TABLE 2. Performances of the HCV Ag and anti-HCV tests compared to the HCV-RNA assay[a]

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	No. of samples		
						Total positive	False negative	False positive
HCV Ag (Abbott)	96.3 (93.3–99.2)	100 (100–100)	100 (100–100)	89.7 (81.8–97.5)	97.2 (93.4–100)	160	6	0
Anti–HCV (Abbott)	96.3 (93.3–99.2)	26.9 (14.9–38.9)	80.2 (74.6–85.8)	70.0 (49.9–90.1)	79.2 (74.9–82.4)	160	6	38

[a] Values in parentheses are ranges. PPV, positive predictive value; NPV, negative predictive value.

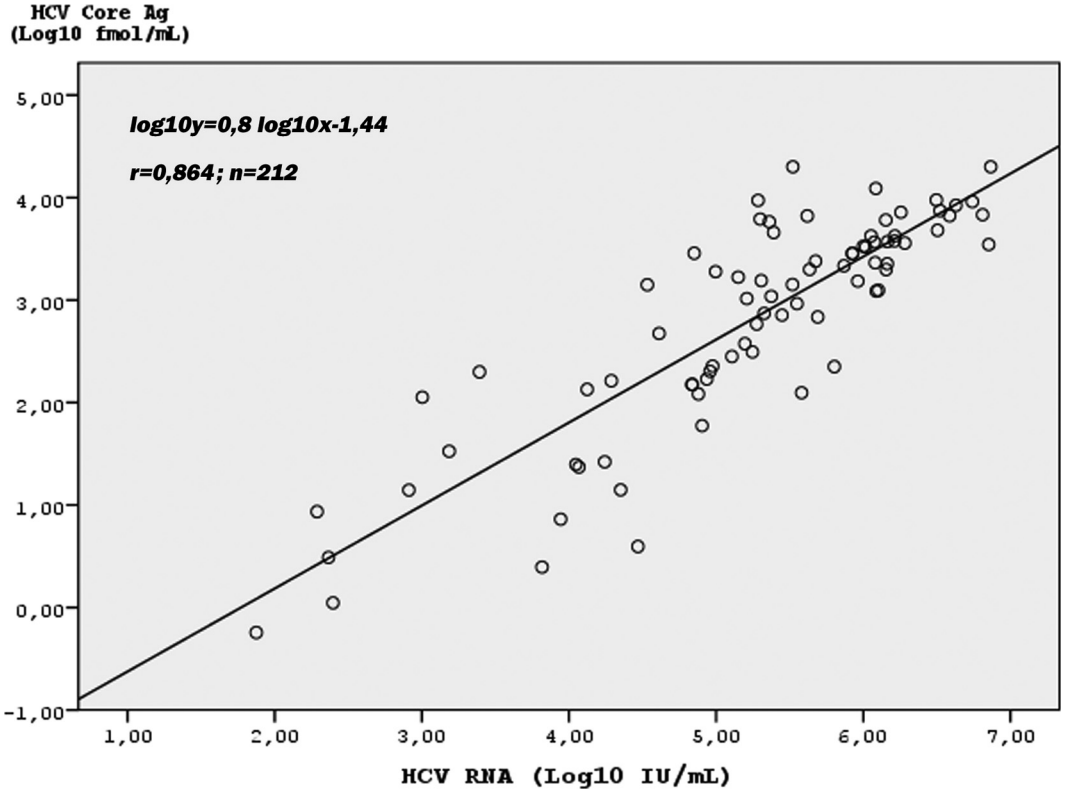

FIG. 1. Correlation between HCV Ag and HCV RNA concentrations. The levels of HCV Ag were highly correlated with those of HCV RNA by the Spearman rank correlation test ($r = 0.864$; $P = 0.0001$). *x*, HCV RNA; *y*, HCV Ag. The data on the axes are log scaled.

grams per milliliter, and *x* is the concentration of HCV RNA in $\log_{10}$ international units per milliliter. This analysis indicated that the relationship between the concentration of HCV Ag and HCV RNA was consistent throughout the common dynamic ranges of the assays.

The sensitivity of the HCV Ag assay was 96.3% (range, 93.3 to 99.2%), and there were no false-positive results or cross-reactivity. The within-run coefficient of variation (CV) was 10%. The levels of HCV Ag showed a good correlation with those from the HCV RNA quantification ($r = 0.907$). The HCV Ag assay showed excellent linearity over the range of 0.5 to 12,000 fmol/liter (Fig. 2).

DISCUSSION

Anti-HCV tests based on CLIA or EIA methods of measuring anti-HCV antibodies in sera are the virologic test methods that are the most commonly used in routine laboratory procedures for the diagnosis of HCV infection worldwide (4, 29). The most common problem in the laboratory-screening anti-HCV assay is the false positivity of samples with low titers. Among immunocompetent populations with an anti-HCV prevalence below 10% (e.g., volunteer blood donors, military personnel, general population, health care workers, or clients attending sexually transmitted diseases clinics), the proportion of false-positive results is approximately 35% (range, 15% to 60%) (14, 21, 24).

Anti-HCV tests have several disadvantages, such as the prolonged duration of the window period between the time of infection and the detection of HCV antibodies: approximately 45 to 68 days. Because anti-HCV tests are based on detection of antibodies that are markers of the immune response, there are many false-negative results in immunocompromised patients because of inadequate observation or a lack of observation of response. Immunoassays also cannot distinguish acute, past, or persistent infections from each other. Patients who have recovered from infection may be found to be seropositive due to the persistence of antibodies (anti-HCV IgG) in serum for a long period and even for life. The anti-HCV test does not distinguish the individuals who have resolved HCV infection from the patients with active/ongoing HCV infection. As a consequence, anti-HCV assay results with values under the critical value used by EIA or CLIA need to be confirmed by an additional confirmatory test, such as the HCV RNA test, or with the preconfirmatory HCV Ag assay (5, 10, 14, 17, 25).

The HCV RNA test is extensively used to confirm antibody-based screening test results. Amplification methods (target amplification by RT-PCR, transmission-mediated amplification [TMA], and signal amplification by branched DNA) are the most expensive methods ($45 to $50 per test for real-time PCR, $10 to $12 per test for HCV Ag CLIA, and $5 to $6 per test for anti-HCV CLIA) compared with anti-HCV and HCV Ag tests and require sophisticated technical equipment and highly trained personnel. One specific problem with the HCV RNA assay is that HCV RNA can be temporarily undetectable because of the transient, partial control of viral replication by the immune response. Patients in a period of

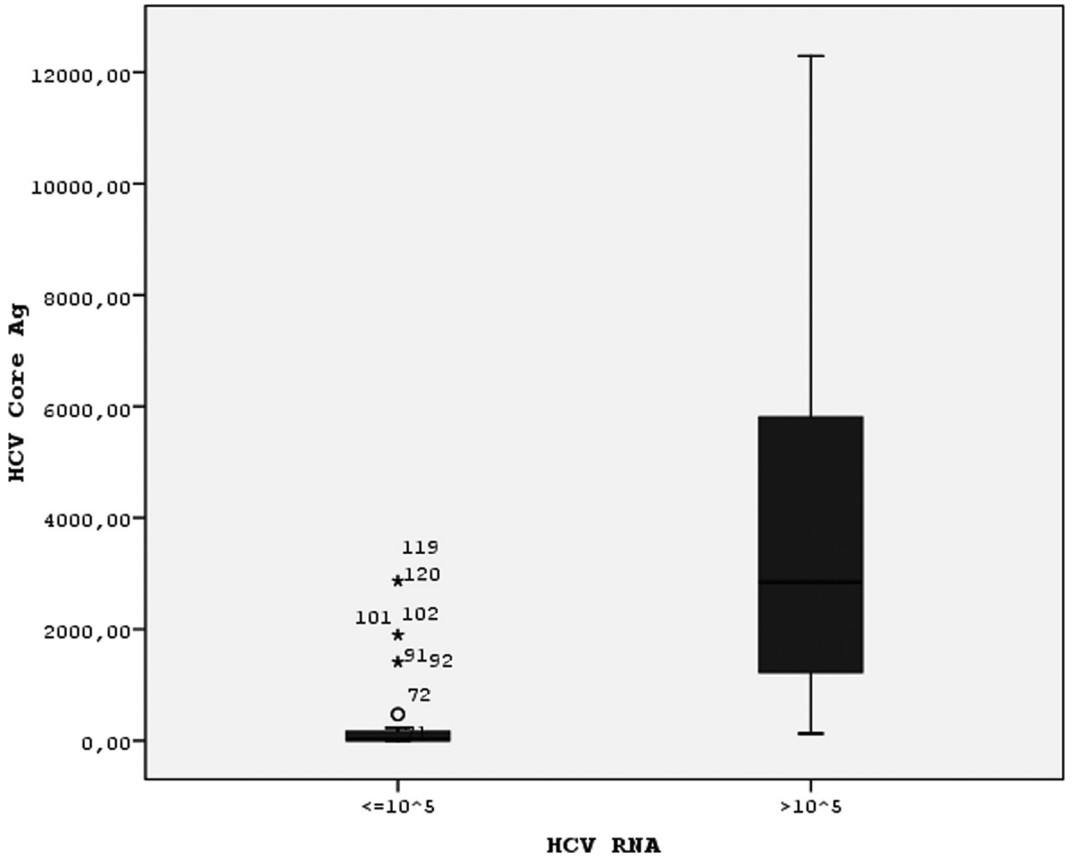

FIG. 2. In the cases in which HCV RNA was detected, the group classifications were arbitrarily made by use of HCV RNA levels of $\leq 10^5$ and $>10^5$ IU/ml (groups A and B, respectively). Median HCV core Ag levels of those groups were 25 and 2848 fmol/liter, respectively, and there were significant differences between them ($P = 0.0001$).

nonviremia may be found to be anti-HCV positive and HCV RNA negative. In this situation, the HCV RNA test should be repeated a few weeks later with a new sample. This need for retesting is a disadvantage of the HCV RNA test. In addition, nucleic acid amplifications are labor-intensive and time-consuming methods and have the risk of laboratory contamination; for these reasons, amplification methods are not suitable for widespread use in most laboratories, especially in developing countries (1, 4, 11, 23, 27). Therefore, the HCV Ag assay is needed as a supplemental or preconfirmatory test to preconfirm anti-HCV results and distinguish false-positive results from the accurate ones because it is easy to perform and reliable, has high specificity and sensitivity rates, is cost-effective, is able to shorten the duration of the time to diagnosis of infection in patients during the window period, and has a lower risk of laboratory contamination than assays based on nucleic acid amplification technology (31, 28).

During the past decade, several HCV Ag tests have been developed as potential alternatives to the HCV RNA assay (3). The first was developed by Tanaka et al. (28) in 1995, and then Aoyagi et al. (2) developed a new and 100-fold more sensitive test in 1999. In previously reported studies, HCV Ag was detected 1 day later than HCV RNA in patients undergoing seroconversion (5, 6, 20).

In this study, the sensitivity, specificity, and positive and negative predictive values of the HCV Ag Abbott CLIA were found to be 96.3%, 100%, 100%, and 89.7%, respectively. In different studies performed with the same test, equal or nearly equal values were found: Park et al. (18) found 100% specificity, Ross et al. (23) found 100% specificity, Morota et al. (16) found 99.8% specificity, Miedouge et al. (15) found 99.2% specificity, Leary et al. (13) found 99% specificity, Song et al. (26) found 97.2% sensitivity, and Park et al. (18) found 90.2% sensitivity. In our study, the diagnostic specificity rate (100%) is equal to the rates from two recent studies performed by Park et al. (18) and Ross et al. (23), almost equal to the value in the study performed by Morota et al. (16) (99.8%), and very near the values from two studies by Miedouge et al. (15) (99.2%) and Leary et al. (13) (99%). The diagnostic sensitivity rate of this study (96.3%) is very close to the rate found by Song et al. (26) (97.2%) and higher than the value found by Park et al. (18) (90.2%).

In this study, the diagnostic specificity and positive predictive value of 100% means that there were no false-positive results: all 154 positive results found by the HCV Ag test were also positive with the HCV RNA test (154/154 subjects). It can be concluded that the positive results of the HCV Ag test can be reported as positive.

In conclusion, the Architect HCV Ag assay is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective, and applicable as a screening, supplemental, and preconfirmatory test for anti-HCV assays in the laboratory procedures used for the diagnosis of hepatitis C virus infection.

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